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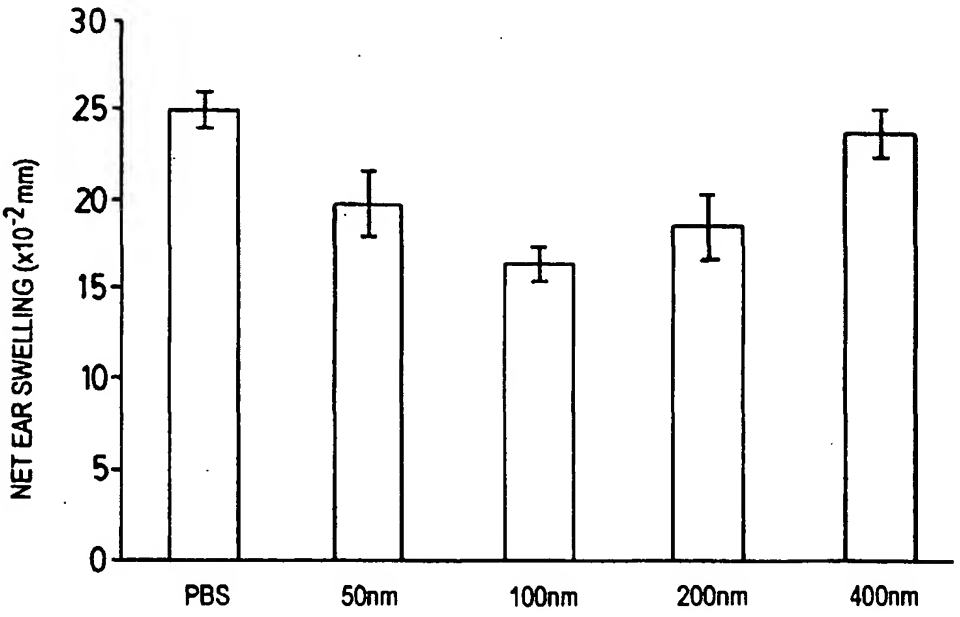
(54) Title: PHARMACEUTICALLY ACCEPTABLE PHOSPHATE-GLYCEROL CARRYING BODIES



(57) Abstract: This invention relates to three-dimensional synthetic and semi-synthetic compositions having biological activity, and to the uses thereof in the treatment and/or prophylaxis of various disorders in mammalian patients. More particularly it relates to preparations and uses of synthetic and semi-synthetic bodies, such as liposomes, which after introduction into the body of a patient, produce beneficial anti-inflammatory, organ protective and immune regulatory effects. The invention also relates to treatments and compositions for alleviating inflammatory and autoimmune diseases and their symptoms.

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PHARMACEUTICALLY ACCEPTABLE PHOSPHATE-GLYCEROL CARRYING BODIES

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to three-dimensional synthetic and semi-synthetic compositions having biological activity, and to the uses thereof in the treatment and/or prophylaxis of various disorders in mammalian patients. More particularly it relates to preparations and uses of synthetic and semi-synthetic bodies, which after introduction into the body of a patient, produce beneficial anti-inflammatory, organ protective and immune regulatory effects. The invention also relates to treatments and compositions for alleviating inflammatory and autoimmune diseases and their symptoms.

Background of the Invention

Professional antigen-presenting cells (APCs), including dendritic cells (DCs) and macrophages (Mph), actively capture and process antigens (Ags), clear cell debris, and remove infectious organisms and dying cells, including the residues from dying cells. During this process, APCs can stimulate the production of either inflammatory Th1 pro-inflammatory cytokines (IL-12, IL-1, INF-γ, TNF-α, etc.); or regulatory Th2/Th3 cytokines (such as IL-10, TGF-β, IL-4 etc.) dominated responses; depending on the nature of the antigen (Ag) or phagocytosed material and the level of APC maturation/activation.

APCs remove cellular debris, some of which is derived from cell membranes of the body, some from bacterial and parasitic infections and commensal organisms, such

as gut bacteria. While some of this cellular debris will initiate a pro-inflammatory response, some initiates a protective and anti-inflammatory response.

A normally functioning immune system is capable of distinguishing between the antigens of foreign invading organisms (non-self) and tissues or debris derived from "self," mounting an immune response only against foreign antigens. When a patient's immune system fails to discriminate between self and non-self, autoimmune disorders arise.

Summary of the Invention

This invention is directed to the discovery that pharmaceutically acceptable bodies, such as liposomes, beads or similar particles, which comprise phosphate-glycerol groups, will, upon administration to a mammalian patient, cause an anti-inflammatory effect and therefore may be used to treat a number of diseases. These bodies may further comprise as a minor component an inactive constituent, and/or constituent which is active through a different mechanism.

In a preferred embodiment, the invention is directed to a composition of matter capable of producing an anti-inflammatory response *in vivo* in a mammal, said composition comprising pharmaceutically acceptable bodies of a size from about 20 nanometers (nm) to 500 micrometers (μm), comprising a plurality of phosphate-glycerol groups or groups convertible to such groups. Preferably, the bodies are essentially free of non-lipid pharmaceutically active entities. Preferably the phosphate-glycerol groups constitute 60% - 100% of the active groups on the bodies. Following administration to a mammal, the bodies, through the phosphate-glycerol groups, are believed to interact with the immune system. As a result, when so administered an anti-inflammatory response is elicited.

In another embodiment, this invention is directed to a three-dimensional synthetic or semi-synthetic body, otherwise referred to herein as pharmaceutically acceptable bodies, having a size ranging from 20 nm to 500 μm, and having been modified to

comprise, as a major component, at least one anti-inflammatory promoting ligand wherein said ligand has phosphate-glycerol groups.

In still another embodiment, this invention is directed to three-dimensional synthetic and semi-synthetic bodies, otherwise referred to herein as pharmaceutically acceptable bodies, having sizes ranging from 20 nm to 500 μm, and having phosphate-glycerol groups on the surface thereof.

In another aspect, the invention is directed to a method for treating a T-cell function-mediated disorder comprising administering to a mammalian patient an effective amount of pharmaceutically acceptable bodies carrying an effective number of phosphate-glycerol groups to inhibit and/or reduce the progression of the T-cell function-mediated disorder.

This invention is further directed to a method for treating an inflammatory disorder comprising administering to a patient an effective amount of pharmaceutically acceptable bodies carrying an effective number of phosphate-glycerol groups to inhibit and/or reduce the progression of the inflammatory disorder.

Yet another embodiment of this invention is a method for treating an endothelial function disorder comprising administering to a mammalian patient an effective amount of pharmaceutically acceptable bodies carrying an effective number of phosphate-glycerol groups to inhibit and/or reduce the progression of the endothelial function disorder.

Another embodiment is a method for treating an immune disorder characterized by inappropriate cytokine expression comprising administering to a mammalian patient an effective amount of pharmaceutically acceptable bodies carrying an effective number of phosphate-glycerol groups to inhibit and/or reduce the progression of the immune disorder.

This invention is further directed to a process for treating or prophylaxis of a mammalian cardiac disorder, the presence of or the susceptibility to which is detectable by observing a prolonged QT-c interval on an electrocardiogram of the patient, which process comprises administering to a mammalian patient suffering therefrom or susceptible thereto a pharmaceutical composition comprising pharmaceutically acceptable biocompatible synthetic or semi-synthetic bodies, otherwise referred to herein as pharmaceutically acceptable bodies, and a pharmaceutically acceptable carrier, wherein at least a portion of said bodies have a size in the range from about 20 nm to 500 μm, and wherein the surfaces of said bodies have been modified to carry, as a major component, at least one anti-inflammatory promoting group, said group being a phosphate-glycerol.

Another embodiment of the invention is a pharmaceutical composition, in unit-dosage form, for administration to a mammalian patient, comprising pharmaceutically acceptable bodies and a pharmaceutically acceptable carrier, wherein at least a portion of the bodies has a size in the range from about 20 nm to 500 μm, and wherein the surfaces of said bodies comprise phosphate-glycerol groups or groups convertible to phosphate-glycerol groups, said unit dosage comprising from about 500 to about 2.5×10^9 bodies.

A further embodiment of this invention is a pharmaceutical composition comprising a pharmaceutically acceptable biocompatible synthetic or semi-synthetic bodies (otherwise referred to herein as pharmaceutically acceptable bodies) and a pharmaceutically acceptable carrier, wherein at least a portion of said bodies has a size from about 20 nm to 500 μm, and wherein the surfaces of said bodies have been modified to comprise, as a major component, at least one anti-inflammatory promoting group, wherein said group is phosphate-glycerol.

A still further embodiment of this invention is a pharmaceutical composition comprising pharmaceutically acceptable biocompatible synthetic or semi-synthetic bodies (otherwise referred to herein as pharmaceutically acceptable bodies) and a

pharmaceutically acceptable carrier, wherein at least a portion of said bodies has a from about 20 nm to 500 μm, and comprises cardiolipin.

Optionally, the bodies described above may additionally comprise an inactive constituent surface group and/or a constituent surface group, which is active through another mechanism, e.g. phosphatidylserine. (See, e.g. Fadok et al., International Publication WO 01/66785).

In another embodiment, this invention is directed to lyophilized or freeze-dried pharmaceutically acceptable bodies carrying phosphate-glycerol groups or groups convertible to phosphate-glycerol groups, and kits comprising lyophilized or freeze dried bodies comprising phosphate-glycerol groups, or groups convertible to phosphate-glycerol groups, and a pharmaceutically acceptable carrier.

In another aspect, this invention is directed to a method for treating a T-cell function-mediated disorder comprising administering to a mammalian patient suffering from or at risk of suffering from a T-cell function mediated disorder, an effective amount of a composition comprising pharmaceutically acceptable bodies having a size from about 20 nm to about 500 μm, comprising on the surface thereof a plurality of phosphate-glycerol groups, or groups convertible to said phosphate-glycerol groups, such that upon administration, the progression of the T-cell function mediated disorder is inhibited and/or reduced.

Yet another embodiment of this invention is directed to a method for treating an endothelial function disorder comprising administering to a mammalian patient suffering from or at risk of suffering from an endothelial function disorder an effective amount of a composition comprising pharmaceutically acceptable bodies having a size of from about 20 nm to about 500 μm, comprising on the surface thereof a plurality of phosphate-glycerol groups, or groups convertible to said phosphate-glycerol groups, such that upon administration, the progression of the endothelial function disorder is inhibited and/or reduced.

Another embodiment of this invention is directed to a method for treating an immune disorder in a mammalian patient suffering from or at risk of suffering from an immune disorder, comprising administering to said mammalian patient an effective amount of a composition comprising pharmaceutically acceptable bodies having a size of from about 20 nm to about 500 μm, comprising on the surface thereof a plurality of phosphate-glycerol groups, or groups convertible to said phosphate-glycerol groups, such that upon administration, the progression of the immune disorder is inhibited and/or reduced.

The present invention can also be viewed, from another aspect, as the use of a receptor on cells of the mammalian immune system, e.g. macrophages, which specifically bind to the phosphate-glycerol group. The invention embraces bodies comprising ligands and groups that will bind to such receptor and consequently produce an anti-inflammatory response. Accordingly, the present invention can be defined as bodies comprising ligands or active groups thereof that compete with the binding or uptake of phosphate-glycerol expressing bodies as described herein by antigen-presenting cells. A person skilled in the art can readily determine whether a particular body is one, which will so compete, by conducting simple test experiments. For example, the bodies can be tested with a readily available monocytic cell line such as U937 cells. In a first experiment, U937 cells are incubated with fluorescently labeled PG liposomes alone, and in other experiments the U937 cells are incubated in the presence of both fluorescently labeled PG liposomes and differing amounts of test compound. If the uptake of the fluorescently labeled PG liposomes in the other experiments is reduced in comparison with that of the first experiment, then the test compound is competing for the specific receptor and is a compound within the scope of the present invention.

Brief description of the drawings

FIG. 1 is a bar graph presentation of the results of Example 1 below, murine contact hypersensitivity (CHS, acute T-cell mediated inflammatory model) experiments using liposomes in accordance with a preferred embodiment of the invention, in

comparison with other liposomes and controls.

FIG. 2 is a similar graphical presentation, showing the use of liposomes of various phosphatidylglycerol (PG) contents, in the murine CHS model, Example 2 below.

FIG. 3 is a similar graphical presentation of the results of Example 3 below where different concentrations of 75% PG liposomes were used in the murine CHS model.

FIG. 4 is a similar graphical presentation of the results of Example 4 below, where different concentrations of 100% PG liposomes were used in the murine CHS model.

FIG. 5 is a similar graphical presentation of the results of Example 5 below, using liposomes of different sizes in the CHS model.

FIG. 6 is a similar graphical presentation of the results of Example 6 below, using a murine model of delayed type hypersensitivity (DHS, chronic T-cell mediated inflammatory model).

FIG. 7 is a similar graphical presentation of the results of Example 7 below, cardiolipin liposomes in a DHS murine model.

FIG. 8 is a similar graphical presentation of the results of Example 8 below, cardiolipin liposomes in a CHS murine model.

FIG. 9 shows the change in the percentage of excitatory post-synaptic potential (EPSP) slope in control and treated mice, which is indicative of the effect on long term potentiation (LTP), Example 9.

FIG. 10 displays the data shown in FIG. 9 in the format of a bar chart, Example 9 below.

FIG. 11 Shows the difference in the concentration of the anti-inflammatory cytokine IL-4 in the hippocampus of control and treated animals, Example 10 below.

FIG. 12 shows the difference in the concentration of the pro-inflammatory cytokine IL-1β in a single cell suspension of spleen cells of control and treated animals, Example 11 below.

FIG. 13 shows the difference in the concentration of TNF-α in the U937 monocyte cell line treated with varying concentration of 75% PG liposomes, Example 12 below.

FIG. 14 is a graphical presentation of the results of Example 13 below, endothelin-1 content in ears of mice treated according to a preferred embodiment of the invention versus control.

FIG. 15 is a graphical presentation of the results of Example 14, ICAM-1 positive cells from HUVEC cultures in the presence and absence of compositions of the preferred embodiment of the invention.

Description of preferred embodiments

According to the present invention, pharmaceutically acceptable bodies carrying phosphate-glycerol groups on their surface are administered to patients. Without being limited to any one theory, is believed that these bodies interact with the immune system of the patient with accompanying beneficial effects such as inhibition of pro-inflammatory cytokines *in vivo* and/or promotion of anti-inflammatory cytokines. The reacting cells may be immune cells such as professional or non-professional antigen presenting cells, endothelial cells, regulatory cells such as NK-T cells and others.

These pharmaceutically acceptable bodies include synthetic and semi-synthetic

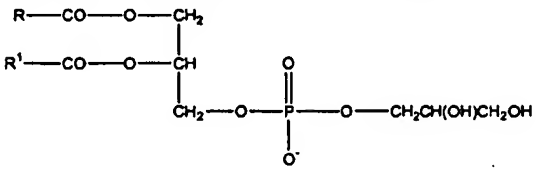
bodies having shapes which are typically but not exclusively spheroidal, cylindrical, ellipsoidal, including oblate and prolate spheroidal, serpentine, reniform etc., and sizes from about 20 nm to about 500 μm in diameter, preferably measured along its longest axis, and comprising phosphate-glycerol groups on the surface thereof.

The pharmaceutically acceptable bodies have phosphate-glycerol groups of predetermined characteristics on the exterior surface. Without being limited to any one theory, it is believed that these groups are capable of interacting with the appropriate receptor(s), other than exclusively the PS receptor, on antigen presenting cells *in vivo*. The structure of these groups may be synthetically altered and include all, part of or a modified version of the original phosphate-glycerol group. For example, the negatively charged oxygen of the phosphate group of the phosphate-glycerol group may be converted to a phosphate ester group (e.g., L-OP(O)(OR')(OR''), where L is the remainder of the phosphate-glycerol group, R' is $-CH_2CH(OH)CH_2OH$ and R'' is alkyl of from 1 to 4 carbon atoms or hydroxyl substituted alkyl of from 2 to 4 carbon atoms, and 1 to 3 hydroxyl groups provided that R'' is more readily hydrolyzed *in vivo* than the R' group; to a diphosphate group including diphosphate esters (e.g., L-OP(O)(OR')OP(O)$(OR'')_2$ wherein L and R' are as defined above and each R'' is independently hydrogen, alkyl of from 1 to 4 carbon atoms, or a hydroxyl substituted alkyl of from 2 to 4 carbon atoms and 1 to 3 hydroxyl groups provided that the second phosphate group [-P(O)$(OR'')_2$] is more readily hydrolyzed *in vivo* than the R' group; or to a triphosphate group including triphosphate esters (e.g., L-OP(O)(OR')OP(O)(OR'')OP(O)$(OR'')_2$ wherein L and R' are defined as above and each R'' is independently hydrogen, alkyl of from 1 to 4 carbon atoms, or a hydroxyl substituted alkyl of from 2 to 4 carbon atoms and 1 to 3 hydroxyl groups provided that the second and third phosphate groups are more readily hydrolyzed *in vivo* than the R' group; and the like. Such synthetically altered phosphate-glycerol groups are capable of expressing phosphate-glycerol *in vivo* and, accordingly, such altered groups are phosphate-glycerol convertible groups.

Phosphatidylglycerol is a known compound. It can be produced, for example, by

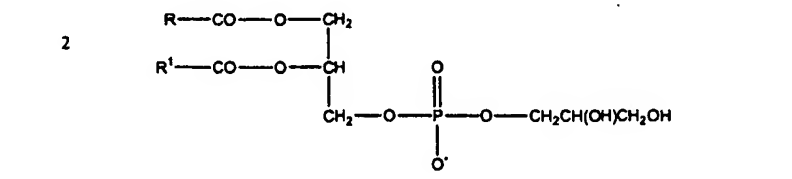
treating the naturally occurring dimeric form of phosphatidylglycerol, cardiolipin, with phospholipase D. It can also be prepared by enzymatic synthesis from phosphatidylcholine using phospholipase D – see, for example, U. S. Patent 5,188,951 Tremblay, et al. Chemically, it has a phosphate-glycerol group and a pair of similar but different C_{18}-C_{20} fatty acid chains.

As used herein the term "PG" is intended to cover phospholipids carrying a phosphate-glycerol group with a wide range of at least one fatty acid chains provided that the resulting PG entity can participate as a structural component of a liposome. Preferably, such PG compounds can be represented by the Formula I:

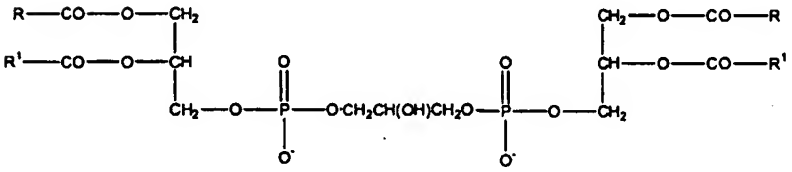


where R and R^1 are independently selected from C_1 – C_{24} hydrocarbon chains, saturated or unsaturated, straight chain or containing a limited amount of branching wherein at least one chain has from 10 to 24 carbon atoms. Essentially, the lipid chains R and R^1 form the structural component of the liposomes, rather than the active component. Accordingly, these can be varied to include two or one such lipid chains, the same or different, provided they fulfill the structural function. Preferably, the lipid chains may be from about 10 to about 24 carbon atoms in length, saturated, mono-unsaturated or polyunsaturated, straight-chain or with a limited amount of branching. Laurate (C12), myristate (C14), palmitate (C16), stearate (C18), arachidate (C20), behenate (C22) and lignocerate (C24) are examples of useful saturated lipid chains for the PG for use in the present invention. Palmitoleate (C16), oleate (C18) are examples of suitable mono-unsaturated lipid chains. Linoleate (C18), linolenate (C18) and arichidonate (C20) are examples of suitable poly-unsaturated lipid chains for use in PG in the liposomes of the present invention. Phospholipids with a single such lipid chain, also useful in the present invention, are known as lysophospholipids. The present invention also extends to cover use of liposomes in which the active component is the dimeric form of PG, namely cardiolipin but other dimers of Formula I are also suitable. Preferably, such dimers

are not synthetically cross-linked with a synthetic cross-linking agent, such as maleimide but rather are cross-linked by removal of a glycerol unit as described by Lehniger, *Biochemistry*, p. 525 (1970) and depicted in the reaction below:



PG



cardiolipin

+

$HOCH_2CH(OH)CH_2OH$

where each R and R^1 are independently as defined above.

As noted above and again without being limited to any one theory, the PG group and its dimer are believed to be a ligand since it is believed that it binds to a specific site on a protein or other molecule ("PG receptor") and, accordingly, this molecule of phosphatidylglycerol (and its dimeric form) is sometimes referred to herein as a "ligand" or a "binding group." Such binding is believed to take place through the phosphate-glycerol group -O-P(=O)(OH)-O-CH_2-CH(OH)-CH_2-OH, which is sometimes referred to herein as the "head group," "active group," or "binding group." In view of the above, reference to "binding," "binding group," or "ligand" herein is not to infer any mechanism or mode of action. Nevertheless, it is believed that the above phosphate-glycerol groups are presented on the exterior surfaces of

the bodies of the present invention for interaction with components of the patient's immune system. This interaction, it should be noted, is not the same as the specific interaction of apoptotic cells with the phosphatidylserine receptor on antigen presenting cells.

Examples of "three-dimensional body portions" or pharmaceutically acceptable bodies" include biocompatible synthetic or semi-synthetic entities such as liposomes, solid beads, hollow beads, filled beads, particles, granules and microspheres of biocompatible materials, natural or synthetic, as commonly used in the pharmaceutical industry. The beads may be solid or hollow, or filled with biocompatible material. The term "biocompatible" refers to substances which in the amount employed are either non-toxic or have acceptable toxicity profiles such that their use *in vivo* is acceptable. Likewise the term "pharmaceutically acceptable" as used in relation to "pharmaceutically acceptable bodies" refers to bodies comprised of one or more materials which are pharmaceutically acceptable and suitable for delivery *in vivo*. Such bodies can include liposomes formed of lipids, one of which is PG. Alternatively, the pharmaceutically acceptable bodies can be solid beads, hollow beads, filled beads, particles, granules and microspheres of biocompatible materials, which comprise one or one or more biocompatible materials such as polyethylene glycol, poly(methylmethacrylate), polyvinylpyrrolidone, polystyrene and a wide range of other natural, semi-synthetic and synthetic materials, with phosphate-glycerol groups attached thereto.

As noted above, analogues of phosphatidylglycerol with modified active groups, which also interact with PG receptors on the antigen presenting cells, through the same receptor pathway as PG or otherwise resulting in an anti-inflammatory reaction in the recipient body are contemplated within the scope of the term phosphatidylglycerol. This includes, without limitation, compounds in which one or more of the hydroxyl groups and/or the phosphate group is derivatized, or in the form of a salt. Many such compounds form free hydroxyl groups in vivo, upon or subsequent to administration and, accordingly, comprise convertible PG groups.

Preferred compositions of matter are liposomes, which may be composed of a variety of lipids. Preferably, however, none of the lipids are positively charged. In the case of liposomes, phosphatidyl glycerol PG may constitute the major portion or the entire portion of the liposome layer(s) or wall(s), oriented so that the phosphate-glycerol group portion thereof is presented exteriorly, to act as the binding group, and the lipid chain or chains form the structural wall.

Liposomes, or lipid vesicles, are sealed sacs, in the micron or sub-micron range, the walls (monolayer or multilayer) of which comprise suitable amphiphiles. They normally contain an aqueous medium, although for the present invention the interior contents are unimportant, and generally inactive. Accordingly, in a preferred embodiment, the liposomes, as well as other pharmaceutically acceptable bodies, are essentially free of non-lipid pharmaceutically active entities (e.g. <1%) and more preferably are free of non-lipid pharmaceutically acceptable entities. Such liposomes are prepared and treated so that the active groups are presented exteriorly on the liposomal body. The PG in the liposomes of the preferred embodiments of this invention thus serves as both a ligand and a structural component of the liposome itself.

Thus a preferred embodiment of this invention provides liposomal bodies which expose or can be treated or induced to expose, on their surfaces, one or more phosphate-glycerol groups to act as binding groups. Phosphatidylglycerol is a preferred PG ligand and such lipids should comprise from 10% - 100% of the liposome, with the balance being an inactive constituent, e.g. phosphatidylcholine PC, or one which acts through a different mechanism, e.g. phosphatidylserine PS, or mixtures of such. Inactive co-constituents such as PC are preferred.

At least 10% by weight of such liposome is composed of PG, preferably at least 50%, more preferably from 60-100% and most preferably from 70-90%, with the single most preferred embodiment being about 75% by weight of PG.

Mixtures of PG liposomes with inactive liposomes and/or with liposomes of phospholipids acting through a different mechanism can also be used, provided that the total amount of PG remains above the minimum of about 10% and preferably above 60% in the total mixture.

As regards to non-liposomal bodies for use in the present invention, these as noted to include biocompatible solid or hollow beads of appropriate size. The biocompatible non-liposomal synthetic or semi-synthetic bodies may be selected from polyethylene glycol, poly(methylmethacrylate), polyvinylpyrrolidone, polystyrene and a wide range of other natural, semi-synthetic and synthetic materials, with phosphate-glycerol groups attached to the surfaces thereof. Such materials include biodegradable polymers, such as disclosed by Dunn, et al. U.S. Patent 4,938,763, which is hereby incorporated by reference in its entirety.

Biodegradable polymers are disclosed in the art and include, for example, linear-chain polymers such as polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers and combinations thereof. Other biodegradable polymers include, for example, gelatin, collagen, etc.

Suitable substances for derivatization to attach the phospholipid(s), or portions thereof with groups or binding groups, to three-dimensional bodies are commercially available e.g. from Polysciences Inc., 400 Valley Road, Warrington, PA 18976, or from Sigma Aldrich Fine Chemicals. Methods for their derivatization are known in the art. Specific preferred examples of such methods are disclosed in International Patent Application PCT/CA02/01398 Vasogen Ireland Limited, which is

incorporated herein by reference.

It is contemplated that the patient may be a mammal, including but not limited to humans and domestic animals such as cows, horses, pigs, dogs, cats and the like.

Phospholipids are amphiphilic molecules (i.e. amphiphiles), meaning that the compound comprises molecules having a polar water-soluble group attached to a water-insoluble hydrocarbon chain. The amphiphiles serving as the layers of the matrix have defined polar and apolar regions. The amphiphiles can include, in addition to PG in this invention, other, naturally occurring lipids used alone with the phospholipid carrying the active group, or in a mixture with another. The amphiphiles serving as the layer(s) of the liposomes can be inert, structure-conferring synthetic compounds such as polyoxyethylene alkylethers, polyoxyethylene alkylesters and saccharosediesters.

Methods of preparing liposomes of the appropriate size are known in the art and do not form part of this invention. Reference may be made to various textbooks and literature articles on the subject, for example, the review article "Liposomes as Pharmaceutical Dosage Forms", by Yechezkel Barenholz and Daan J. A. Chrommelin, and literature cited therein, for example New, R. C. "Liposomes: A Practical Approach", IRL Press at Oxford University Press (1990).

The diameter of the liposomes, as well as the other pharmaceutically acceptable bodies, of the preferred embodiment of this invention is from about 20 nm to about 500 μm, more preferably from about 20 nm to about 1000 nm, more preferably from about 50 nm to about 500 nm, and most preferably from about 80 nm to about 120 nm (preferably measured along its longest axis). In one embodiment, the diameter of the liposome is from 60nm to 500μm.

The pharmaceutically acceptable bodies may be suspended in a pharmaceutically acceptable carrier, such as physiological sterile saline, sterile water, pyrogen-free

water, isotonic saline, and phosphate buffer solutions (e.g. sterile aqueous solutions comprising phosphate buffer), as well as other non-toxic compatible substances used in pharmaceutical formulations, such as, for example, adjuvants, buffers, preservatives, and the like. Preferably, the pharmaceutically acceptable bodies are constituted into a liquid suspension in a sterile biocompatible liquid such as buffered saline and administered to the patient by any appropriate route which exposes it to one or more components of the immune system, such as intra-arterially, intravenously or most preferably intramuscularly or subcutaneously.

It is contemplated that the pharmaceutically acceptable bodies may be freeze-dried or lyophilized so that they may be later resuspended for administration. This invention is also directed to a kit of part comprising lyophilized or freeze-dried binding group-carrying bodies and a pharmaceutically acceptable carrier, such as physiological sterile saline, sterile water, pyrogen-free water, isotonic saline, and phosphate buffer solutions (e.g. sterile aqueous solutions comprising phosphate buffer), as well as other non-toxic compatible substances used in pharmaceutical formulations, such as, for example, adjuvants, buffers, preservatives, and the like. Protectants for freeze drying, as known in the art, for example lactose or sucrose, may also be included.

A preferred manner of administering the pharmaceutically acceptable bodies to the patient is a course of injections, administered daily, several times per week, weekly or monthly to the patient, over a period ranging from a week to several months. The frequency and duration of the course of the administration is likely to vary from patient to patient, and according to the condition being treated, its severity, and whether the treatment is intended as prophylactic, therapeutic or curative. Its design and optimization is well within the skill of the attending physician. Intramuscular injection, especially via the gluteal muscle, is most preferred. One particular injection schedule, in at least some of the indications of the invention, is an injection, via the gluteal muscle, of an appropriate amount of bodies on day 1, a further injection on day 2, a further injection on day 14, and then "booster"

injections at monthly intervals, if appropriate.

It is postulated that, in many embodiments of the present invention, pharmaceutically acceptable bodies comprising the PG groups as binding groups on their surface are acting as modifiers of the patient's immune system, in a manner similar to that of a vaccine. Accordingly they are used in quantities and by administration methods to provide a sufficient localized concentration of the bodies at the site of introduction. Quantities of such bodies appropriate for immune system modification may not be directly correlated with body size of a recipient and can, therefore, be clearly distinguished from drug dosages, which are designed to provide therapeutic levels of active substances in a patient's bloodstream and tissues. Drug dosages are accordingly likely to be much larger than immune system modifying dosages.

The correlation between weights of liposomes and numbers of liposomes is derivable from the knowledge, accepted by persons skilled in the art of liposomal formulations, that a 100 nm diameter bilayer vesicle has 81,230 lipid molecules per vesicle, distributed approximately 50:50 between the layers (see Richard Harrigan – 1992 University of British Columbia PhD Thesis "Transmembrane pH gradients in liposomes (microform): drug-vesicle interactions and proton flux", published by National Library of Canada, Ottawa, Canada (1993); University Microfilms order no. UMI00406756; Canadiana no. 942042220, ISBN 0315796936). From this one can calculate, for example, that a dose of 5×10^8 vesicles, of the order of the dose used in the specific *in vivo* examples below, is equivalent to 4.06×10^{13} lipid molecules. Using Avogadro's number for the number of molecules of lipid in a gram molecule (mole), 6.023×10^{23}, one determines that this represents 6.74×10^{-11} moles which, at a molecular weight of 729 for PG is approximately 3.83×10^{-8}gm, or 38.3 ng of PG for such dosage.

The quantities of the pharmaceutically acceptable bodies to be administered will vary depending on the nature of the mammalian disorder it is intended to treat and

on the identity and characteristics of the patient. Preferably, the effective amount of pharmaceutically acceptable bodies is non-toxic to the patient, and is not so large as to overwhelm the immune system. When using intra-arterial, intravenous, subcutaneous or intramuscular administration of a sterile aqueous suspension of pharmaceutically acceptable bodies, it is preferred to administer, for each dose, from about 0.1-50 ml of liquid, containing an amount of bodies generally equivalent to 10% - 1000% of the number of leukocytes normally found in an equivalent volume of whole blood. Preferably, the number of bodies administered per delivery to a human patient is in the range from about 500 to about 2.5×10^9 (<250 ng of bodies, in the case of liposomes, pro-rated for density differences for other embodiments of bodies), more preferably from about 1,000 to about 1,500,000,000, even more preferably 10,000 to about 50,000,000, and most preferably from about 200,000 to about 2,000,000.

Since the pharmaceutically acceptable bodies are acting, in the process of the invention, as immune system modifiers, in the nature of a vaccine, the number of such bodies administered to an injection site for each administration maybe a more meaningful quantitation than the number or weight of bodies per unit of patient body weight. For the same reason, it is now contemplated that effective amounts or numbers of bodies for small animal use may not directly translate into effective amounts for larger mammals (i.e. greater than 5 kg) on a weight ratio basis.

The present invention is indicated for use in prophylaxis and/or treatment of a wide variety of mammalian disorders where T-cell function, inflammation, endothelial dysfunction and inappropriate cytokine expression are involved. A patient having or suspected of having such a disorder may be selected for treatment. "Treatment" refers to a reduction of symptoms, such as, but not limited to, a decrease in the severity or number of symptoms of the particular disease or a limit on the further progression of symptoms.

With respect to T-cell function (T-cell mediated) disorders, these disorders include

any and all disorders mediated at least in party by T-cells and include for example, ulcers, wounds, and autoimmune disorders including, but not limited to diabetes, scleroderma, psoriasis and rheumatoid arthritis.

The invention is indicated for use with inflammatory allergic reactions, organ and cell transplantation reaction disorders, and microbial infections giving rise to inflammatory reactions. It is also indicated for use in prophylaxis against oxidative stress and/or ischemia reperfusion injury, ingestion of poisons, exposure to toxic chemicals, radiation damage, and exposure to airborne and water-borne irritant substances, etc., which cause damaging inflammation. It is also indicated for inflammatory, allergic and T-cell-mediated disorders of internal organs such as kidney, liver, heart, etc.

With respect to disorders involving inappropriate cytokine expression for which the present invention is indicated, these include any and all disorders involving inappropriate cytokine expression and include, for example, neurodegenerative diseases. Neurodegenerative diseases, including Down's syndrome, Alzheimer's disease and Parkinson's disease, are associated with increased levels of certain cytokines, including interleukin-1β (IL-1β) (see Griffin WST et al. (1989); Mogi M. et al. (1996)). It has also been shown that IL-1β inhibits long-term potentiation in the hippocampus (Murray, C. A. et al. (1998)). Long-term potentiation in the hippocampus is a form of synaptic plasticity and is generally considered to be an appropriate model for memory and learning (Bliss, T.V.P. et al. (1993)). Thus, inappropriate cytokine expression in the brain is currently believed to be involved in the development and progression of neurodegenerative diseases and neuroinflammatory disorders.

Thus, the invention is indicated for the treatment and prophylaxis of a wide variety of mammalian neurodegenerative and other neurological disorders, including Downs syndrome, Alzheimer's disease, Parkinson's disease, senile dementia, depression, Huntingdon's disease, peripheral neuropathies, Guillain Barr syndrome, spinal cord

diseases, neuropathic joint diseases, chronic inflammatory demyelinating disease, neuropathies including mononeuropathy, polyneuropathy, symmetrical distal sensory neuropathy, neuromuscular junction disorders, myasthenias and amyotrophic lateral sclerosis (ALS). Treatment and prophylaxis of these neurodegenerative diseases represents a particularly preferred embodiment of the invention, with treatment of Alzheimer's disease, Parkinson's disease and ALS particularly preferred.

Regarding disorders involving endothelial dysfunction, the present invention is indicated for the treatment and prophylaxis of a wide variety of such mammalian disorders including, any and all disorders mediated at least in part by endothelial dysfunction and include, for example, cardiovascular diseases, such as atherosclerosis, peripheral arterial or arterial occlusive disease, congestive heart failure, cerebrovascular disease (stroke), myocardial infarction, angina, hypertension, etc., vasospastic disorders such as Raynaud's disease, cardiac syndrome X, migraine etc., and the damage resulting from ischemia (ischemic injury or ischemia-reperfusion injury). In summary, it can be substantially any disorder the pathology of which involves an inappropriately functioning endothelium.

Further indications for the compositions and processes of the present invention include the treatment of patients to accelerate their rate of wound healing and ulcer healing, and treatment of patients prior to surgical operations, to accelerate their rate of recovery from surgery including their rate of healing of surgical wounds and incisions.

In regard to "cardiac disorders," the present invention is indicated for the treatment and prophylaxis of a wide variety of such mammalian disorders including, any and all disorders relating to the heart and include, for example, ventricular arrhythmias (ventricular tachycardia or fibrillation) and sudden death from heart disease. Susceptibility of patients to cardiac disorders such as arrhythmias and sudden cardiac death is often indicated by prolonged QT-c intervals in the heart beat

rhythm. Administration of compositions according to the preferred embodiments of the invention is believed to reduce QT-c intervals in mammalian patients, indicative of reduced susceptibility of to arrhythmia and sudden cardiac death.

The invention is further described, for illustrative purposes, in the following non-limiting examples.

EXAMPLES

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has it generally acceptable meaning.

μg	=	microgram
μL	=	microliter
μm	=	micrometer
μM	=	micromolar
CHS	=	contact hypersensitivity
cm	=	centimeter
DMSO	=	dimethylsulfoxide
DNFB	=	2,4-dinitrofluorobenzene
DHS	=	delayed-type hypersensitivity
EtOH	=	ethanol
g	=	gram
hrs	=	hours
Hz	=	hertz
IM	=	intramuscular
IP	=	intraperitoneal
kg	=	kilogram
LPS	=	lipopolysaccharide
LTP	=	long-term potentiation
mg	=	milligram
min	=	minutes

ml	=	milliliter
mM	=	millimolar
ms	=	millisecond
ng	=	nanogram
nm	=	nanometer
nM	=	nanomolar
PBS	=	phosphate-buffered saline
PCR	=	polymerase chain reaction
POPS	=	1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[phospho-L-serine], referred to herein as PS
POPG	=	1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[phospho-rac-(1-glycerol)]], referred to herein as PG
POPC	=	1-Palmitoyl-2-Oleoyl-sn-Glycero-3-phosphocholine, referred to herein as PC
RPM	=	revolutions per minute
S	=	second

Unless otherwise stated, the precise form of the lipids used in the experiments was POPS, POPG and POPC as set out above.

Example 1

Liposomes of 100 ± 20 nm in average diameter were prepared according to standard methods known in the art and had the following compositions:

Group A - 100% PS

Group B - 100% PG

Group C – control, no liposomes.

A stock suspension of each liposome composition containing 4.8×10^{14} liposomes per ml was diluted with PBS to give an injection suspension containing 6×10^{6} particles per ml. The liposomal suspensions were injected into female BALB/c mice (Jackson Laboratories) aged 6-8 weeks and weighing 19-23 g, to determine the

effect on ear swelling in the murine contact hypersensitivity (CHS) model. The CHS model tests for Th1-mediated inflammatory reactions.

The animals were assigned to one of 3 groups, with 5 animals in each group. Groups A and B received approximately 3 x 10^5 of the above-identified liposomes (*i.e.*, 100% PC and 100% PG, respectively), in a volume of approximately 50 μl. Group C was a control group, receiving no liposomes.

Protocol

The following experiments were performed:

TABLE I

Group	Lipo-somes	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (24 hours)
A	100% PS	Injected then sensitized	Injected	Injected	Injected	Injected	Injected then challenged	Ear measured
B	100% PG	Injected then sensitized	Injected	Injected	Injected	Injected	Injected then challenged	Ear measured

On Days 1-6, mice of Groups A and B were injected with the respective liposomes preparations. Approximately 300,000 liposomes were injected in 50 μl volume via intramuscular (IM) injection, for a total administration over the test period of about 1,800,000 liposomes. Mice of the control group (Group C) received no liposomes,

but were sensitized, challenged and tested in the same way as Groups A and B, as described below.

Sensitization

On Day 1, following liposome injection for that day, mice were anaesthetized with 0.2 ml 5 mg/ml sodium pentobarbital via IP injection. The abdominal skin of the mouse was sprayed with 70% EtOH and a scalpel blade was used to remove about a one-inch diameter patch of hair from the abdomen. The shaved area was then painted with 25 μl of 0.5% 2,4-dinitrofluorobenzene (DNFB) in 4:1 acetone: olive oil using a pipette tip.

Challenge

Following liposome injection on day 6, mice were challenged with DNFB by painting 10 μl of 0.2% DNFB on the dorsal surface of the right ear with a pipette tip and by painting 10 μl of vehicle on the left ear with a pipette tip.

Results

On Day 7, 24 hours after challenge, each animal was anaesthetized with Halothane, and ear thickness was measured using a Peacock spring-loaded micrometer. Data was expressed as the difference between the treated right ear thickness and the thickness of the vehicle-treated left ear. The experiments were repeated three times, on similar animals. Increase in ear swelling was used as a measure of CHS response. The significance of the data was determined by the two-tailed student's t-test. A P value of <0.05 was considered significant.

The results are presented in FIG. 1, a bar graph showing the mean values from the three experiments of ear swelling, reported in μm.

FIG. 1 shows that a significant reduction in ear swelling was achieved by injection of liposomes according to the present invention. The reduction achieved with 100%

PG liposomes is substantially greater than that from 100% PS liposomes.

Example 2

Liposomes of 100 ± 20 nm in average diameter were prepared according to standard methods known in the art and had the following compositions:

Group A – 100% PG
Group B – 75% PG, 25% PC
Group C – 50% PG, 50% PC
Group D – 25% PG, 75% PC
Group E – PBS only
Group F – no injection

A stock suspension of each liposome containing 4.8×10^{14} liposomes per ml was diluted to give an injection suspension containing 12×10^{6} liposomes per ml. The liposomal suspensions were used to inject into mice to determine the effect on ear swelling in the murine CHS model, a biological system useful for assaying Th1-mediated inflammatory reactions. For these experiments, female BALB/c mice (Jackson Laboratories) aged 6-8 weeks and weighing 19-23 g were used.

The animals were assigned to one of 6 groups (Groups A-F, above) with 10 animals in each group. Control groups were also included that received no injections (Group F) or injections of PBS with no liposomes (Group E). Animals in Groups A-D were injected with 50µl of the above-identified liposome suspensions, each containing about 6×10^{5} liposomes.

Protocol

The test involves sensitization (Sens) with a potentially inflammation-causing substance, injection of liposomes (Inj) in test animals or PBS in controls and

challenge (Chal) with the potentially inflammation-causing substance following measurement (Meas) to determine whether the injection of liposomes are effective against the development of inflammation by the challenge.

The following experiments were performed:

Group	Lipo-somes	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A	100% PG	Sens & Inj	Inj	Inj	Inj	Inj	Chal & Inj	Meas
B	75% PG	Sens & Inj	Inj	Inj	Inj	Inj	Chal & Inj	Meas
C	50% PG	Sens & Inj	Inj	Inj	Inj	Inj	Chal & Inj	Meas
D	25% PG	Sens & Inj	Inj	Inj	Inj	Inj	Chal & Inj	Meas
E	None (PBS only)	Sens & Inj	Inj	Inj	Inj	Inj	Chal & Inj	Meas
F	none	Sens					Chal	Meas

On days 1-6 the mice were injected with the respective liposomes as indicated above. Liposomes were injected in 50µl volume via IM injection, *i.e.*, 600,000 liposomes per injection, for a total administration over the test period of 3,600,000 liposomes. Mice of the control group received no liposomes but were sensitized, challenged and tested in the same way as the other groups of mice, as described below.

Sensitization (Sens)

On Day 1, following liposome injection for that day, mice were anaesthetized with 0.2 ml 5 mg/ml sodium phenobarbital via IP injection. The abdominal skin of the

mouse was sprayed with 70% EtOH and a blade was used to remove about a one inch diameter of hair from the abdomen. The bare area was painted with 25 µl of 0.5% 2,4-dinitrofluorobenzene (DNFB) in 4:1 acetone: olive oil using a pipette tip.

Challenge (Chal)

On Day 6, following liposomes injection for that day, mice were challenged (Chal) with DNFB as follows: 10 µl of 0.2% DNFB was painted on the dorsal surface of the right ear with a pipette tip and 10 µl of vehicle was painted on the left ear with a pipette tip.

Results

On Day 7, 24 hours after challenge, each animal was anaesthetized with Halothane, and ear thickness was measured (Meas) using a Peacock spring-loaded micrometer. Increase in ear swelling was used as a measure of CHS response. Data was expressed as the difference in the treated right ear thickness minus the thickness of the vehicle treated left ear. The significance between the two groups is determined by a two-tailed student's t-test. A P value of <0.05 is considered significant.

The results are presented graphically in FIG. 2, a bar graph showing ear swelling in µm. The mean value from the respective experiments was used in compiling the graph.

FIG. 2 shows that a significant reduction in ear swelling with both 100 and 75% PG is achieved, showing that both these concentrations protect against the development of inflammation resulting from contact with the allergenic substance, DNFB. The 50% and the 25% PG liposomes also showed reductions as compared with both controls, but the differences did not reach statistical significance in this experiment.

Example 3

Liposomes of 100 ± 20 nm in average diameter were prepared according to standard methods known in the art and were composed of 75% PG, 25% PC. A stock suspension containing 4.8 x 10^{14} liposomes per ml was used as before and diluted in PBS to give an injection suspension containing the following concentrations of liposomes:

Group	Liposomes	Concentration (liposomes per mL)	Liposomes per injection	Animals in Group
A	75% PG, 25% PC	12 x 10^{11}	6 x 10^{10}	10
B	75% PG, 25% PC	12 x 10^{9}	6 x 10^{8}	10
C	75% PG, 25% PC	12 x 10^{8}	6 x 10^{7}	16
D	75% PG, 25% PC	12 x 10^{7}	6 x 10^{6}	16
E	75% PG, 25% PC	12 x 10^{6}	6 x 10^{5}	16
F	none (PBS only)			16

BALB-c mice were divided into six groups (Groups A-F) including a control group receiving no liposomes but injected with 50 μL of PBS (Group F). Mice were sensitized on the flank, injected with their selected liposomal dose, intramuscularly to the right leg muscle, on the same day as, but after, sensitisation (day 1) and on days 2, 3, 4, and 5. On day 6 they were both injected and challenged on the ear as described in Example 1. The thickness of the ear was measured as described 24 hours after the challenge.

The results (FIG. 3) show a significant difference between the control group (Group F) and Group C (12×10^8 liposomes per ml) and between the control group and Group D (12×10^7 liposomes per ml) and between the control group and Group E (12×10^6 liposomes per ml). There was little difference between the control group and Groups A or B (12×10^{11} and 12×10^9 liposomes per ml, respectively), suggesting that there is an optimum range of liposome concentrations above which the beneficial effects may be reduced. In other experiments, a decrease in effect was also be observed as the concentration of the liposomes was decreased below 12×10^4 liposomes per ml.

Example 4

Liposomes of formulation 100% PG and 100±20 nm in average size were prepared according to standard methods. Four groups (Groups A-D) of 10 mice were sensitised, injected and challenged in accordance with the procedure and schedule described in Example 3, with the following numbers of 100% PG liposomes delivered in a 50 µl suspension.

Group A – 6×10^7

Group B – 6×10^6

Group C – 6×10^5

Group D – 6×10^4

The results, along with the PBS control from Example 4, are presented in similar bar graph form in FIG. 4. A significant reduction in ear swelling, as compared with the control group is to be noted for each of the test groups, but with little difference between the various groups.

Example 5

Liposomes of composition 75% PG, 25% PC and of 50, 100, 200, of 400 nm in average diameter were prepared by standard methods. They were tested in the murine CHS model, as in Examples 3 and 4, using 6×10^5 liposomes in 50 µl suspensions for each injection, and a sensitisation-injection-challenge schedule and

procedure as in Example 3. The groups were as follows:

Group A	-	50 nm liposomes
Group B	-	100 nm liposomes
Group C	-	200 nm liposomes
Group D	-	400 nm liposomes
Group E	-	no liposomes

The results are presented in FIG. 5. The result from Group D, using the 400 nm diameter liposomes, is not significantly different from the control group (Group E), indicating a probable size range criticality in this model.

Example 6

A stock suspension of 75% PG liposomes of 100±20 nm in average diameter containing 4.8×10^{14} liposomes per ml was diluted to give an injection suspension containing 6×10^5 liposomes per ml. The liposomal suspensions were used to inject into mice, to determine the effect on ear swelling in the murine DHS model. As in Example 1, female BALB/c mice (Jackson Laboratories) aged 6-8 weeks and weighing 19-23 g were used.

The animals were assigned to one of 3 groups with 10 animals in each group. A control group (Group C) received only PBS injections. Animals of Groups A and B were injected with 50 µl of a suspension containing 6×10^5 liposomes.

Protocol

On days 13-18 the mice were injected with the 75% PG liposomes as indicated below. Liposomes were injected in 50 µl volume via IM injection, *i.e.*, 600,000 liposomes per injection, for a total administration over the test period of 3,600,000 liposomes. Sensitization and challenge took place as described in Example 2.

DAY	TREATMENT

1	Sensitized
6	Challenged
7	Measured
12	Challenged
13	Measured & Injected
14	Injected
15	Injected
16	Measured & Injected
17	Injected
18	Injected & Challenged
19	Measured

Results

The results are presented graphically in accompanying FIG. 6 and show that 75% PG is effective in the DHS model on day 16, 24 hours after the third injection following the second challenge.

Example 7

Liposomes of composed of 100% cardiolipin (CL) and 100±20 nm in average diameter were prepared, by standard methods. These were used at a dosage of 6 x 10^5 liposomes per 50 µl per injection in the murine DHS model described in Example 6. Data obtained from animals injected with CL liposomes (Group A; 10 animals) was compared to data obtained from animals receiving only PBS (Group B; 10 animals). The sensitisation, injection and challenge procedures were as described in Example 2. The ear thickness measurement results, taken on day 19, 24 hrs after the 6^{th} injection, are presented in FIG. 7. The results showed a significant reduction in ear swelling within the CL-injected test (Group A).

Example 8

Liposomes of 100 nm in average diameter, and comprising either 100% cardiolipin or 75% cardiolipin and 25% PC, were prepared by standard methods. Three groups (Groups A-C) of 10 mice were sensitised on day 1. A control group received injections of PBS on days 1, 2 and 6 (Group C). The other two groups received injections, of 6×10^5 100% cardiolipin liposomes (Group A) or of 6×10^5 75% cardiolipin liposomes (Group B), liposomes in 50 µl per injection according to the same schedule. The mice were challenged on day 7, and the ear thickness measured, as described in the previous examples.

FIG. 8 shows the mean measurements in each group. Both groups receiving CL liposomes showed a statistically significant suppression of CHS compared to the control group

Example 9

To study the cellular and molecular mechanisms underlying cognitive function, the Long-Term Potentiation (LTP) animal model is used. LTP is a form of synaptic plasticity that occurs in the hippocampal formation, which has been proposed as a biological substrate for learning and memory (Bliss *et al.* Nature 361:31-39 (1990)). LTP in rats is monitored electrophysiologically by methods well known to those in the art. The animals are then sacrificed to investigate biochemical changes in hippocampal tissues. Comparing the results of electrophysiological data with biochemical hippocampal changes is useful for determining how the cellular events that underlie LTP may be altered in animals suffering from diseases or disorders associated with neuroinflammation such as aging, stress, Alzheimer's disease, and bacterial infection.

Systemic administration of lipopolysaccharide (LPS), a cell-wall component of Gram-negative bacteria, provokes an activation of the immune system by inducing an increase in pro-inflammatory cytokines such as IL-1β. As noted above, one

example of a neuronal deficit induced by LPS and IL-1β is the impairment of LTP in the hippocampus. An indicator of LTP is the mean slope of the population excitatory post-synaptic potential (epsp). Upon tetanic stimulation, the epsp slope (%) increases sharply indicating increased synaptic activity. LPS-induced inhibition of LTP reduces the increase in slope, and/or causes the epsp slope to revert more rapidly to base line, indicating that the increased synaptic activity is short-lived. Accordingly measurements of the epsp slope (%) at timed intervals after tetanic stimulation can be used to reflect memory and the loss thereof following an inflammatory stimulus as well as inflammation in the hippocampus of the brain.

Liposomes of 100±20 nm in average diameter were prepared as according to standard methods known in the art and were composed of 75% PG and 25% PC. A stock suspension of the liposomes containing about 2.9×10^{14} liposomes per ml was diluted with PBS to give an injection suspension containing about 1.2×10^{7} liposomes per ml. This was then used to inject into rats, to determine the effect on LPS-induced impairment of LTP. For these experiments, male Wistar rats (BioResources Unit, Trinity College, Dublin), weighing approximately 300 g, were used.

The animals were assigned to one of four groups, 8 animals in each group to be treated as follows:

Group A - saline + control
Group B - saline + PG
Group C - LPS + control
Group D - LPS + PG

150 µl of each above-identified preparation was injected via IM injection on days 1, 13, and 14. Groups B and D received a total of 5,400,000 liposomes (1,800,000 liposomes per injection). The LTP procedure and tissue preparation procedure were carried out on day 0.

LTP Procedure

Rats were anaesthetized by IP injection of urethane (1.5 g/kg). Rats received either LPS (100 μg/kg) or saline intraperitoneally. Three hours later a bipolar stimulating electrode and a unipolar recording electrode were placed in the perforant path and in the dorsal cell body region of the dentate gyrus respectively. Test shocks of 0.033 Hz were given and responses recorded for 10 min before and 45 min after high frequency stimulation (3 trains of stimuli delivered at 30 s intervals, 250 Hz for 200 ms).

Rats were killed by decapitation. The hippocampus, the tetanized and untetanized dentate gyri, the cortex and entorhinal cortex were dissected on ice, sectioned and frozen in 1 ml of Krebs solution (composition of Krebs in mM: NaCl 136, KCl 2.54, KH_2PO_4 1.18, $MgSO_4.7H_2O$ 1.18, $NaHCO_3$ 16, glucose 10, $CaCl_2$ 1.13) containing 10% DMSO.

Results

The results are shown in FIG. 9. The graph shows the difference in the excitatory post-synaptic potential (epsp) recorded in cell bodies of the granule cells. The data presented are means of seven to eight observations in each treatment group and are expressed as mean percentage change in epsp slope every 30 s normalized with respect to the mean value in the 5 minutes immediately prior to tetanic stimulation. FIG. 9 shows that the LPS-induced inhibition of LTP in perforant path-granule cell synapses was overcome by pre-treatment with the PG liposomes. The filled triangles represent Group A (saline + control), the open triangles represent Group B (saline + PG), the filled squares represent Group C (LPS + control) and the open squares represent Group D (LPS + PG).

FIG. 10 shows that analysis of the mean values 40-45 minutes post tetanic stimulation indicate that the population epsp slope was decreased in the control-LPS group (open bars) and that the PG liposomes (hashed bars) significantly reversed this effect (*$p<0.01$). As an index of memory and learning functionality, the

improvement in sustainability of LTP demonstrated in this Example indicates suitability of the treatment for dementias e.g. Alzheimer's disease and memory impairment.

Example 10

IL-4 is one of a number of cytokines secreted by the Th2 subclass of lymphocytes and is known for its anti-inflammatory effects. FIG. 11 shows that the IL-4 concentration in the hippocampus was significantly increased in the LPS group that had been pre-treated with the PG liposomes (*$p<0.05$). Open bars represent control group (Group E) and hashed bars represent the PG treated group (Group F). IL-4 was measured by ELISA and expressed as PG of IL-4 per mg of total protein. This upregulation of the anti-inflammatory cytokine IL-4 in the brain is indicative of the use of the process and composition of preferred embodiments of the present invention in treating a wide range of neuroinflammatory disorders, including Parkinson's disease, ALS, chronic inflammatory demyelinating disease CIPD and Guillain Barr syndrome.

Example 11

IL-1β is one of a number of cytokines secreted by the Th1 subclass of lymphocytes and is known for its proinflammatory effects. Spleens from animals treated as described in Example 9, groups C and D thereof, were extracted and spleen cells collected. They were prepared as follows:

FIG. 12 shows that the IL-1β concentration in spleen cells was significantly reduced in the LPS group that had been pre-treated with the PG liposomes (*$p<0.05$). IL-1β was measured by ELISA and expressed as picagrams of IL-1β per mg of total protein. This indicates a systemic inflammatory effect of the process and compositions of preferred embodiments of the present invention.

Example 12

U937 is a monocytic leukemia cell line that can be differentiated into macrophages

by administration of phorbol esters. Treatment with lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, stimulates an inflammatory response in U937 cells, with the upregulation of expression of a number of inflammatory molecules including TNFα. This model provides an experimental system for the assessment of anti-inflammatory therapies. The macrophages can be grown in culture medium in the presence of a suspected anti-inflammatory composition, and the expression of TNFα can be measured.

Liposomes of 100 ± 20 nm in average diameter were prepared according to standard methods known in the art and had a composition of 75% phosphatidylglycerol (PG), 25% phosphatidylcholine (PC). The stock concentration of liposome was about 40 mM lipid and was diluted to the following final concentrations in the assay:
100 μM phosphatidylglycerol (PG)
40μM PG
10 μM PG
4.0 μM PG
1 μM PG

The U937 cells were cultured by growing in RPMI medium (GIBCO BRL) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and grown at 37°C in an atmosphere containing 5% CO_2. 5 x 10^5 cells were seeded into wells of 6-well plates and caused to differentiated into macrophages by treatment with 150 nM phorbol myristate acetate (PMA) for 2-3 days. The cell medium was then replaced with complete medium after the U937 cells had differentiated into macrophages. The cells were then incubated for an additional 24 hrs to minimize pleotropic effects due to PMA treatment.

The cells were then incubated with either:

Group A Phosphate buffered saline (PBS) – as a negative control,
Group B 10 ng/ml LPS – as a positive control,
Group C 10 ng/ml LPS + 100 μM PG,

Group D	10 ng/ml LPS + 40 μM PG,
Group E	10 ng/ml LPS + 10 μM PG,
Group F	10 ng/ml LPS + 4.0 μM PG, or
Group G	10 ng/ml LPS + 1 μM PG.

The cells were incubated as described above at 37°C in 5% CO_2. After 18 hrs, the supernatants from each treatment were collected and assayed for TNF-α using a standard Quantikine Enzyme-Linked Immunosorbent Assay (ELISA) kit (R&D systems, Minneapolis, USA).

Results

FIG. 13 shows the amount of secreted TNF-α in PG per ml. The results demonstrates that U937-differentiated macrophage cells express very low levels of TNF-α under normal conditions. However, once exposed to LPS, they secrete large amounts of TNF-α into the surrounding medium, which is indicative of cellular stress occurring. Incubation of the cells with PG liposomes inhibits the secretion of TNF-α in a dose-dependent manner, with the highest concentration of 100μM resulting in a 98% decrease, and even the lowest concentration of 1μM causing a 58% decrease in TNF-α expression.

Example 13

To determine the effect of the PG liposomes of the preferred embodiment of the present invention on endothelial function, the endothelin-1 (ET-1) content in the ears of mice which had been subjected to the CHS studies as described in Example 3 was determined. Endothelin-1 is a potent vasoconstrictive agent, has inotropic and mitogenic actions, modulates salt and water homeostasis and plays an important role in the maintenance of vascular tone and blood pressure. Various lines of evidence indicate that endogenous ET-1 may contribute to the pathophysiology of conditions associated with sustained vasoconstriction, such as heart failure. In heart failure, elevated levels of circulating ET-1 and big-ET-1 are observed (Giannessi D, Del Ry

S, Vitale RL. "The role of endothelins and their receptors in heart failure." *Pharmacol Res* 2001 Feb 43:2 111-26). Thus ET-1 is a marker of endothelial function and increased production of ET-1 in tissue is indicative of impaired endothelial function.

In order to determine ET-1 expression, mouse ears (right challenged ear) were harvested 24 hrs after challenge in CHS experiments. Ears were obtained from mice injected intramuscularly with PBS for 6 days (Group A) and mice injected intramuscularly with 75% PG/25% PC liposomes (600,000 liposomes/injection; Group B). Ears were stored in RNAlater at –20°C until RNA extraction. RNA was extracted and cDNA was generated using reverse transcriptase (RT) along with ET-1-specific primers, as an internal control, PCR was also performed using ∃-actin-specific primers. PCR products were resolved on a 1.5% agarose gel and the DNA bands were quantitated by densitometry analysis. The ratio of ET-1/β-actin was calculated.

PCR Preparation:

PCR Mix (ET-1)	PCR Mix (β-Actin)
5µl PCR Buffer (10x)	5µl PCR Buffer (10x)
1.5µl MgCl2 (50mM)	1.5µl MgCl2 (50mM)
1µl dNTP (10mM)	1µl dNTP (10mM)
0.5µl Primer 1 (25uM)	1 µl Primer 1 (10uM)
0.5µl Primer 2 (25uM)	1µl Primer 2 (10uM)
0.25µl TAQ	0.25µl TAQ
2.5µl cDNA	2.5µl cDNA
38µl Water	37.75µl Water
50µl Total	50µl Total

Primers : (as previously described – see for example Yang, L; Husain, M; and Stewart. D.J., **"Conditional cardiac overexpression of endothelin-1 in transgenic mice"**, FASEB J 15(5):A1138 – A1138 Part 2, MAR8 2001.

ET-1(r) 5'-CAG CAC TTC TTG TCT TTT TGG-3'
ET-1(f) 5'-CCA AGG AGC TCC AGA AAC AG-3'

β-Actin(F) 5'-GTG GGC CGC TCT AGG CAC CAA-3'
β-Actin(r) 5'-CTC TTT GAT GTC ACG CAC GAT TTC-3'

PCR Settings:

94°C – 5minutes

94°C – 30s
60°C – 30s } 30 cycles
72°C – 60s

72°C – 10 minutes

4°C - Soak

After 6-daily injections of the 75% PG liposomes, the level of ET-1 was decreased by 36% relative to control mice receiving PBS during the same injection regimen. The results are shown graphically on FIG. 14. This decrease indicates a beneficial effect resulting from the injection of the liposomes of the preferred embodiment of the invention on endothelial function in a mammalian patient, through Th1 mediated inflammation reduction.

Example 14

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface molecule expressed by several cell types, including leukocytes and endothelial cells. It is involved in the adhesion of monocytes to endothelial cells and plays a role in inflammatory processes and in the T-cell mediated host defense system. ICAM-1 expression probably contributes to the clinical manifestations of a variety of diseases, predominantly by interfering with normal immune function. Among these are malignancies (e.g., melanoma and lymphomas), many inflammatory disorders (e.g., asthma and autoimmune disorders), atherosclerosis, ischemia, certain neurological disorders, and allogeneic organ transplantation (Van de Stolpe A, van der Saag PT, "Intercellular adhesion molecule-1" *J. Mol. Med.* (1996) 74:1 13-33).

Human umbilical vein endothelial cells (HUVECs) are a primary cell line of endothelial cells that are isolated from umbilical vein cords as follows.

T75 flasks were prepared by coating with 0.2% gelatin (5-7 ml/flask) for a minimum of 15/20minutes or overnight. The excess was then removed. The cord was sprayed with 70% ethanol prior to procedure and any placenta still remaining attached to the cord was cut away. The cord was then cut to an approximate length of 5-6 inches. The cord has two arteries which are thick walled and one vein that is bigger and thin walled. The vein was located and the serrated edge of a stopper placed into it. Approximately 20cm of string was then used to tie the cord onto the stopper.

The cord was then washed through with phosphate buffered saline (PBS) a number of times until the PBS ran clear. Following this 15-20mls of Collagenase solution was placed into the cord; it was wrapped in tinfoil and incubated for 15minutes at 37°C. After incubation the tied end of the cord was cut and the collagenase drained into a 50ml tube. Collagenase was then passed through the cord again, the cord was massaged to loosen the endothelial cells and then PBS was passed through the cord

and collected into the same tube containing the collagenase solution. This was then centrifuged at 1600RPMs, the supernatant removed and the pellet resuspended in 10-12 mls of M199 complete medium. Finally the medium containing the cells was added to the gelatinized flasks.

Liposomes of 100 ± 20 nm in average diameter were prepared according to standard methods known in the art and had a composition of 75% phosphatidylglycerol (PG), 25% phosphatidylcholine (PC). The stock concentration of liposome was 40 mM lipid and was diluted to 100 μM in the assay.

HUVECs split into a number of tissue culture flasks, allowed to adhere to the surface of the flask and then treated as follows:
Group A - PBS – as a negative control,
Group B - 500 ng/ml LPS – as a positive control,
Group C - 500 ng/ml LPS + 100 μM PG
Group D - 500 ng/ml LPS + 100 μM PC

The cells were incubated at 37°C, 5% CO_2. After 18 hrs, the supernatants from each treatment were collected and assayed for ET-1 using a standard ELISA kit (obtained from Assay Designs) and the cells harvested for analyzing ICAM-1 as follows.

The cells were first washed with PBS and then incubated with a cell dissociation buffer at 37°C for 25-30 min. The cells were then washed by centrifugation and incubated with an anti-CD54 (ICAM-1) antibody for 30 minutes. A secondary FITC antibody was then added and incubated with the cells as before. Finally they were resuspended in 1 ml of PBS and analyzed for fluorescence on a flow cytometer.

Results

The results are presented on FIG. 15, a graphical presentation of the percentage of cells staining positive for ICAM-1 in the respective cultures. It is to be noted that the numbers of cells staining positive in the PG liposome-containing culture is

reduced to negative control level, and is much lower than the positive control level.

Example 15

Microglial cells (brain macrophages) were cultured, and their output of TNF-α, an inflammatory cytokine, was measured. The cells were stimulated with the immunoglobulin (IgG) of patients suffering from ALS, and the TNF-α output increased about 800-fold as a result. When the same cells were grown in the presence of both the ALS IgG and PG liposomes, output of TNF-α decreased by about 75%, indicating the potential of the preferred embodiments of the present invention in the treatment of ALS. The results are presented in FIG. 16.

What is claimed is

1. A composition of matter capable of producing an anti-inflammatory response *in vivo* in a mammal, said composition comprising pharmaceutically acceptable bodies of a size from about 20 nanometers (nm) to 500 micrometers (μm), comprising a plurality of phosphate-glycerol groups or groups convertible to phosphate-glycerol groups.

2. The composition according to claim 1, wherein the bodies are liposomes.

3. The composition according to Claim 2, wherein said composition is essentially free of non-lipid pharmaceutically acceptable entities.

4. The composition according to Claim 2, wherein said composition is free of non-lipid pharmaceutically acceptable entities.

5. The composition according to Claim 2, Claim 3 or Claim 4, wherein the liposomes comprise from about 60 to 100% of phosphatidylglycerol (PG).

6. The composition according to Claim 5, wherein the liposomes comprise from about 70% - 90% of phosphatidylglycerol.

7. The composition according to Claim 5 or Claim 6, wherein the remainder of the liposome comprises phosphatidylcholine.

8. The composition according to any of claims 2 – 7 wherein the liposomes have a size from about 50 – 500 nanometers.

9. The composition according to claim 8 wherein the liposomes have a size from about 80 – 120 nanometers.

10. For use in the manufacture of a medicament for the treatment of a T-cell function-mediated disorder, a composition comprising pharmaceutically acceptable biocompatible bodies having a size in the range of from about 20 nm to 500 μm and having expressed or expressible on the surface a plurality of PG head groups.

11. For use as claimed in claim10, wherein the PG head groups are expressed on the surface of the bodies and are head groups of PG ligands.

12. For use as claimed in claim 10 or claim 11, wherein the bodies are liposomes.

13. For use as claimed in claim 12, wherein the liposomes are constituted by 50% - 100% by weight of phosphatidylglycerol

14. For use as claimed in claim 13, wherein the liposomes are constituted by 65% -90% by weight of phosphatidylglycerol.

15. For use as claimed in claim 11, claim 12 or claim 13, wherein the liposomes have a diameter of from about 20 nanometers to about 1000 nanometers

16. For use as claimed in any of claims 10 – 15, a unit dosage of said composition comprising from about 50 to about 2.5 x 10^9 bodies.

17. For use as claimed in claim 16, a unit dosage of said composition comprising from about 10,000 to about 50,000,000 bodies.

18. For use in the manufacture of a medicament for the treatment of an inflammatory disorder, a composition comprising pharmaceutically acceptable biocompatible bodies having a size in the range of from about 20

nm to 500 μm and having expressed or expressible on the surface a plurality of PG head groups.

19. For use as claimed in claim18, wherein the PG head groups are expressed on the surface of the bodies and are head groups of PG ligands.

20. For use as claimed in claim 18 or claim 19, wherein the bodies are liposome.

21. For use as claimed in claim 20, wherein the liposomes are constituted are constituted by 50% - 100% by weight of phosphatidylglycerol.

22. For use as claimed in claim 21, wherein the liposomes are constituted by 65% -90% by weight of phosphatidylglycerol.

23. For use as claimed in claim 20, claim 21 or claim 22, wherein the liposomes have a diameter of from about 20 nanometers to about 1000 nanometers.

24. For use as claimed in any of claims 20 – 23, a unit dosage of said composition comprising from about 50 to about 2.5 x 10^9 bodies.

25. For use in the manufacture of a medicament for the treatment of an endothelial function disorder, a composition comprising pharmaceutically acceptable biocompatible bodies having a size in the range of from about 20 nm to 500 μm and having expressed or expressible on the surface a plurality of PG head groups.

26. For use as claimed in claim 25, wherein the PG head groups are expressed on the surface of the bodies and are head groups of PG ligands.

27. For use as claimed in claim 25 or claim 26, wherein the bodies are liposomes.

28. For use as claimed in claim 27, wherein the liposomes are constituted are constituted by 50% - 100% by weight of phosphatidylglycerol

29. For use as claimed in claim 28, wherein the liposomes are constituted by 65% -90% by weight of phosphatidylglycerol.

30. For use as claimed in claim 27, claim 28 or claim 29, wherein the liposomes have a diameter of from about 20 nanometers to about 1000 nanometers.

31. For use as claimed in any of claims 27 - 30, a unit dosage of said composition comprising from about 50 to about 2.5 x 10^9 bodies.

32. For use as claimed in claim 31, a unit dosage of said composition comprising from about 10,000 to about 50,000,000 bodies.

33. For use as claimed in claim 32, a unit dosage of said composition comprising from about 10,000 to about 50,000,000 bodies.

34. For use in the manufacture of a medicament for the treatment of an immune disorder characterized by inappropriate cytokine expression, a composition comprising pharmaceutically acceptable biocompatible bodies having a size in the range of from about 20 nm to 500 μm and having expressed or expressible on the surface a plurality of PG head groups.

35. For use as claimed in claim 34, wherein the PG head groups are expressed on the surface of the bodies and are head groups of PG ligands.

36. For use as claimed in claim 34 or claim 35, wherein the bodies are liposomes.

37. For use as claimed in claim 36, wherein the liposomes are constituted are constituted by 50% - 100% by weight of phosphatidylglycerol.

38. For use as claimed in claim 37, wherein the liposomes are constituted by 65% -90% by weight of phosphatidylglycerol.

39. For use as claimed in claim 36, claim 37 or claim 38, wherein the liposomes have a diameter of from about 20 nanometers to about 1000 nanometers.

40. For use as claimed in any of claims 36 - 39, a unit dosage of said composition comprising from about 50 to about 2.5×10^9 bodies.

41. For use as claimed in claim 40, a unit dosage of said composition comprising from about 10,000 to about 50,000,000 bodies.

42. For use as claimed in claim 41, a unit dosage of said composition comprising from about 10,000 to about 50,000,000 bodies.

43. For use as claimed in any of claims 34 – 42, wherein the disorder is a neurological disorder.

44. A pharmaceutical composition, in unit-dosage form, for administration to a mammalian patient, comprising pharmaceutically acceptable bodies and a pharmaceutically acceptable carrier, wherein at least a portion of the bodies has a size in the range from about 20 nm to 500 μm, and wherein the surfaces of said bodies comprise phosphate-glycerol groups or groups convertible to phosphate-glycerol groups, said unit dosage comprising from about 500 to about 2.5×10^9 bodies.

45. The pharmaceutical composition of Claim 44 wherein the bodies are liposomes.

46. The pharmaceutical composition of Claim 45, wherein the composition is essentially free of non-lipid pharmaceutically acceptable entities.

47. The pharmaceutical composition of Claim 45, wherein the composition is free of non-lipid pharmaceutically acceptable entities.

48. The pharmaceutical composition of Claim 45, Claim 46 or Claim 47 wherein the liposome comprises phosphate-glycerol groups comprise from about 60 to 100% of phosphatidylglycerol, PG.

49. The pharmaceutical composition of Claim 48, wherein the liposome comprises from 70 – 90% PG.

50. The pharmaceutical composition of Claim 48 or claim 49, wherein any remainder of the liposome is phosphatidylcholine.

51. The pharmaceutical composition of claim 44 wherein said bodies comprise surface groups which specifically bind to receptors on cells of the mammalian immune system specific for the binding of phosphate-glycerol groups.

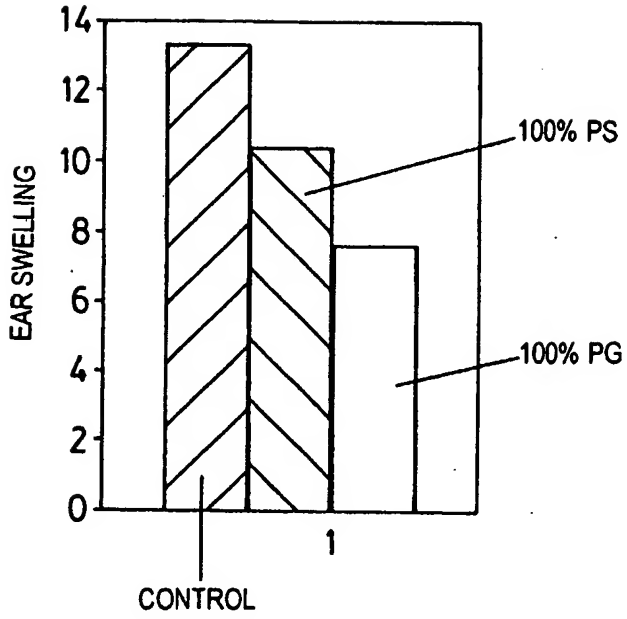


FIG. 1

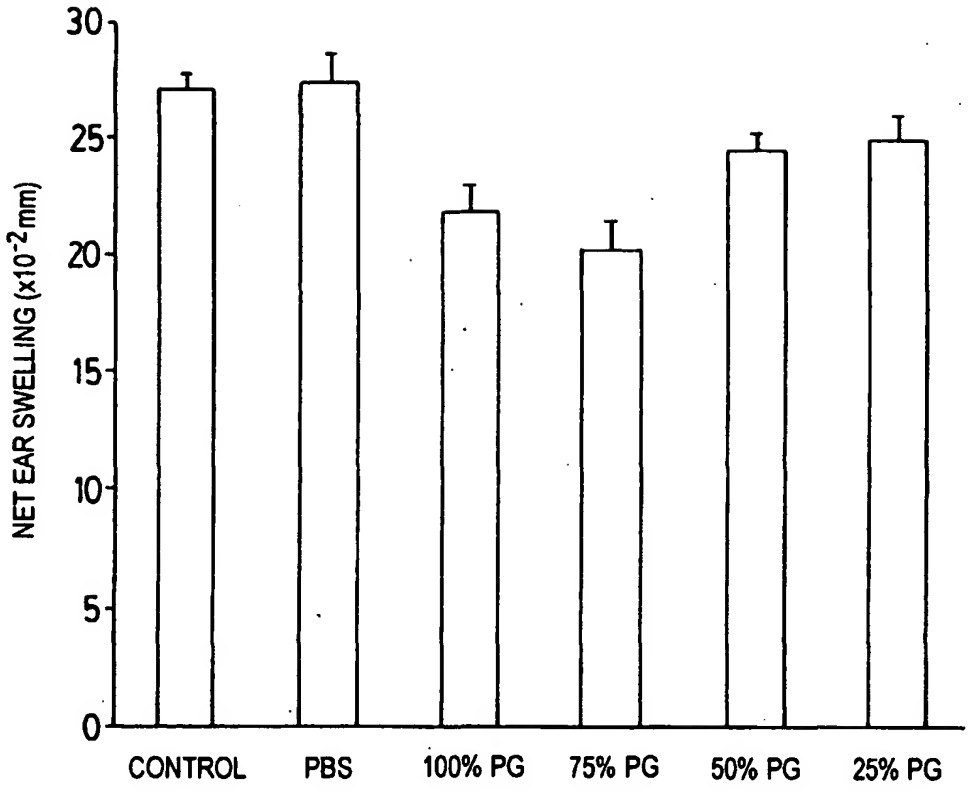

30
25
20
15
10
5
0
NET EAR SWELLING (x10^{-2}mm)
CONTROL
PBS
100% PG
75% PG
50% PG
25% PG

FIG. 2

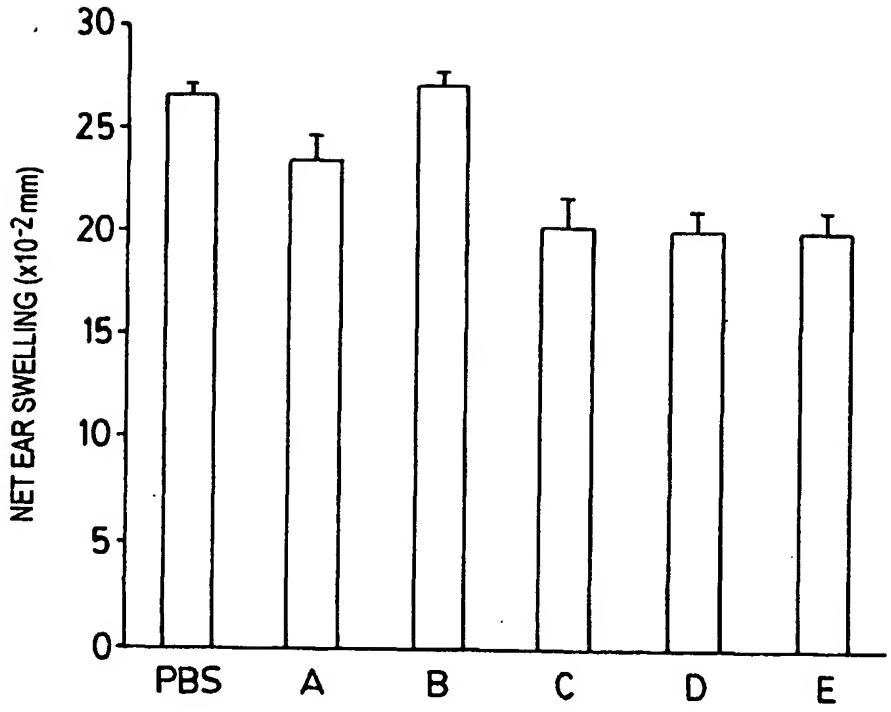


FIG. 3

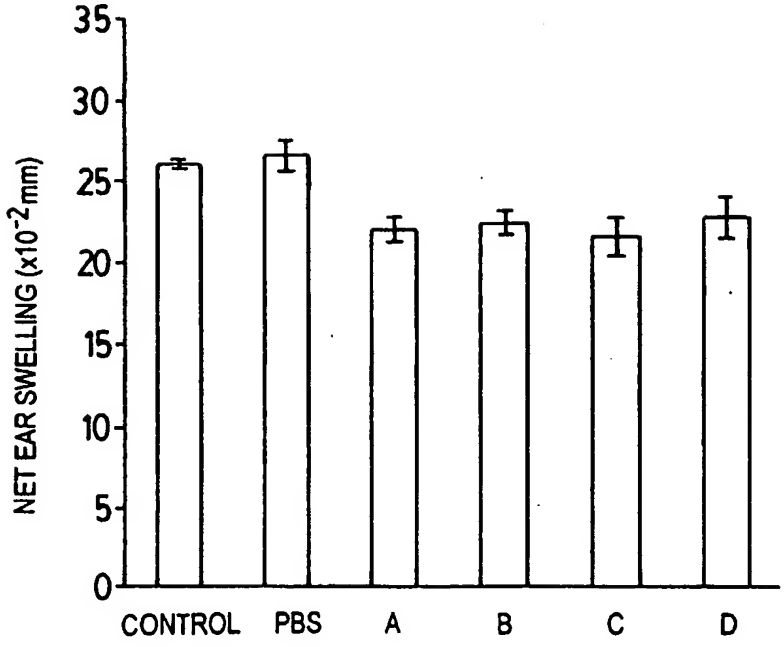

NET EAR SWELLING (x10^{-2}mm)
35
30
25
20
15
10
5
0
CONTROL
PBS
A
B
C
D

FIG. 4

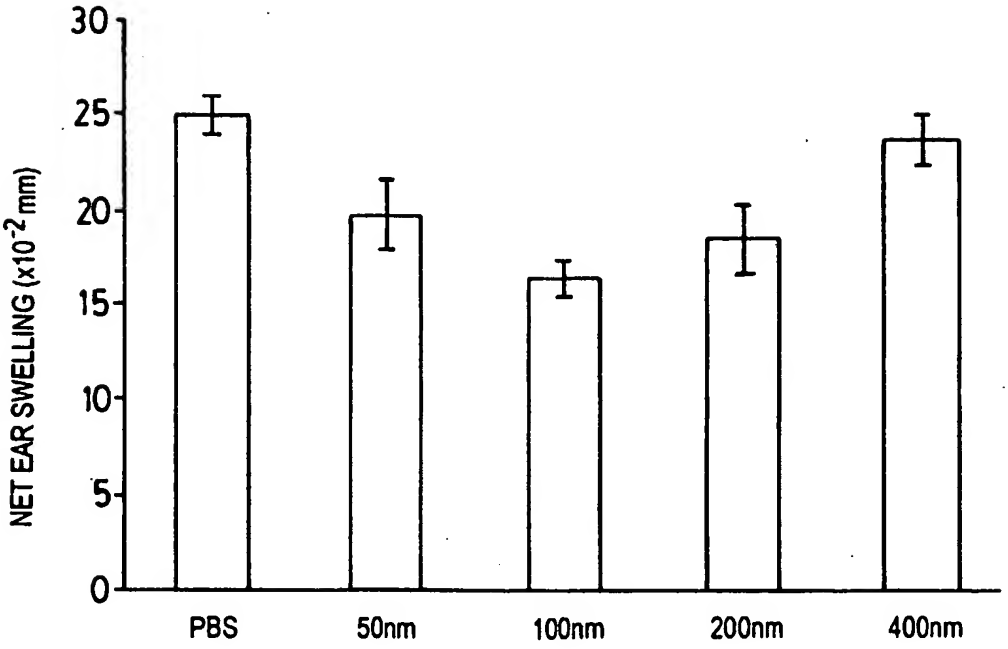

NET EAR SWELLING (x10^{-2}mm)
30
25
20
15
10
5
0
PBS
50nm
100nm
200nm
400nm

FIG. 5

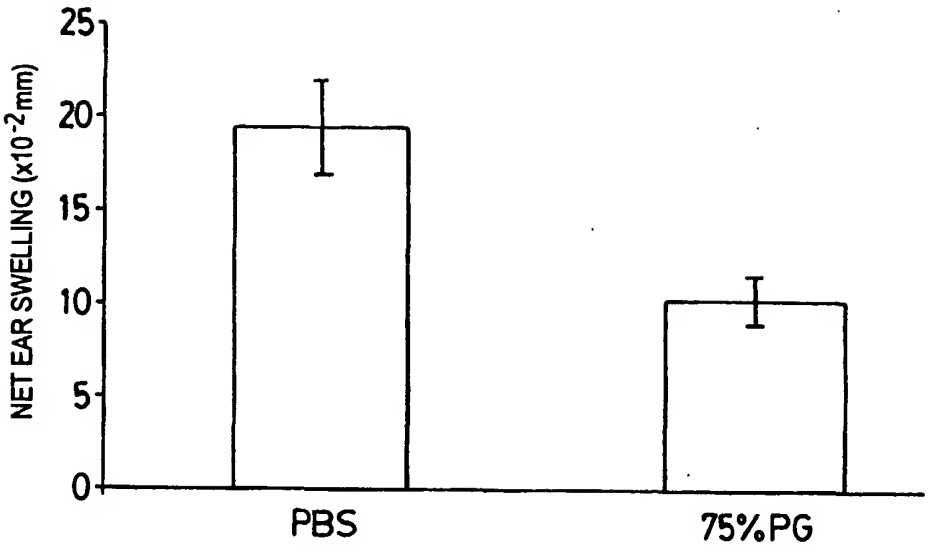


FIG. 6

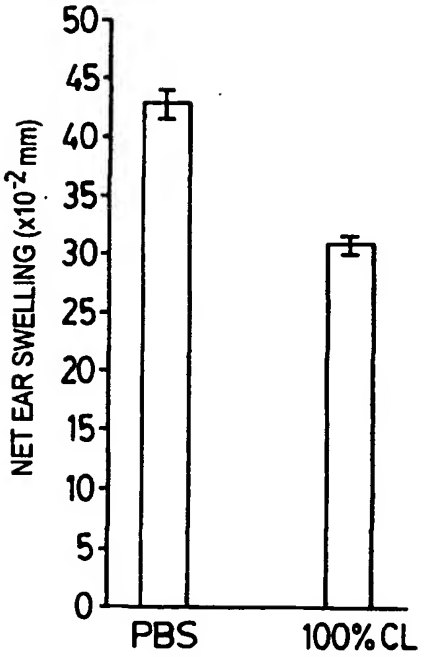


FIG. 7

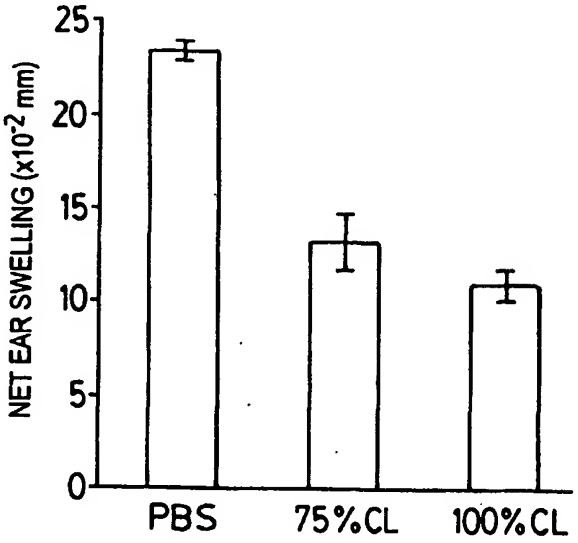


FIG. 8

LTP (EPSP SLOPE) IN PERFORANT PATH-GRANULE CELL SYNAPSES – EFFECT OF PG

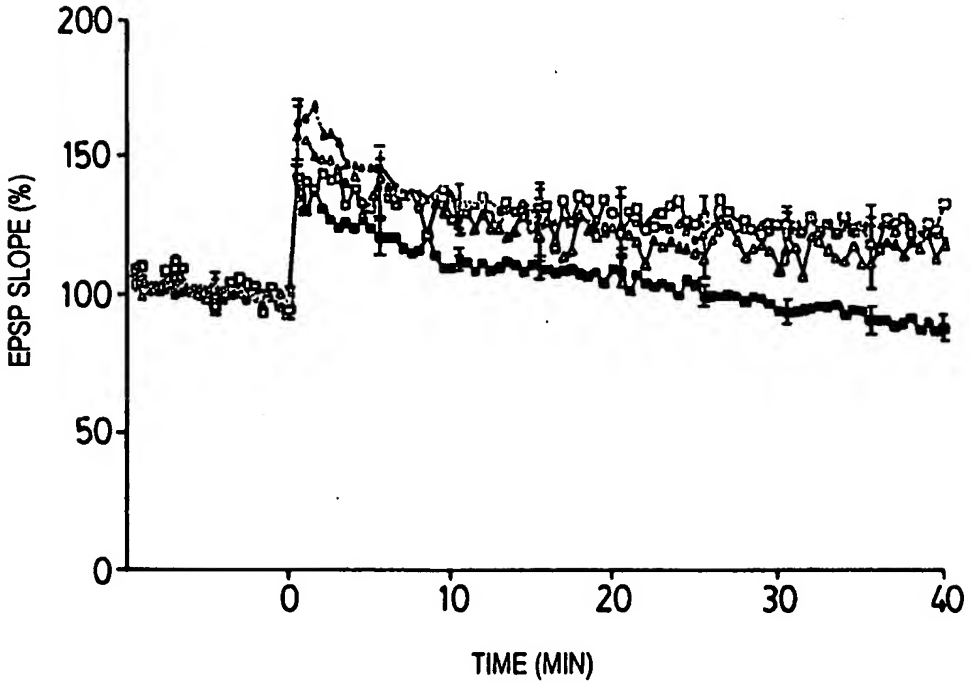
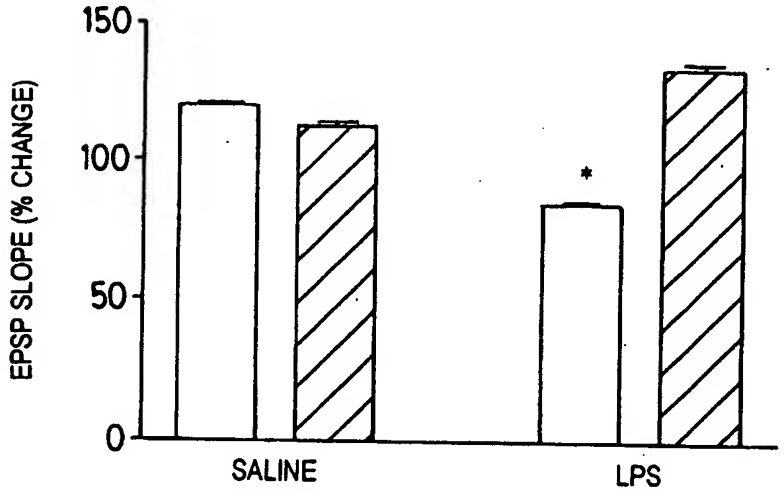


FIG. 9

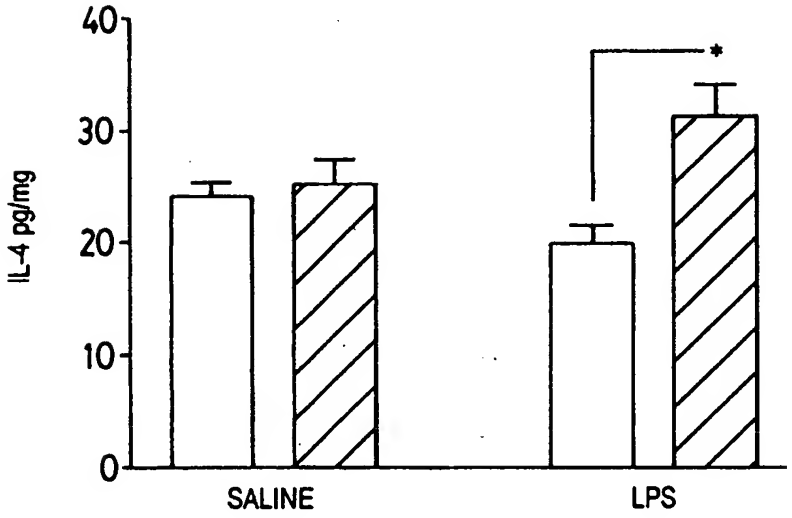
LTP (EPSP SLOPE) IN PERFORANT PATH-GRANULE CELL SYNAPSES – 40-45 MIN POST-HFS



DATA EXPRESSED AS MEANS WITH STANDARD ERRORS *p<0.01 STUDENT'S T-TEST

FIG. 10

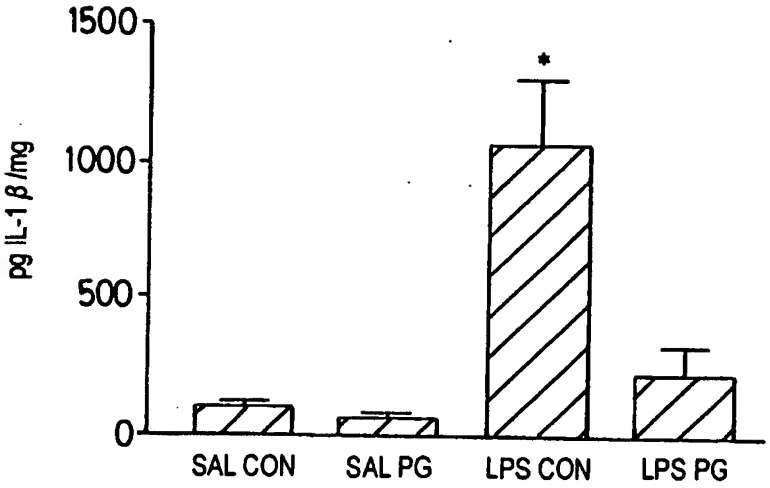
IL-4 CONCENTRATION IN HIPPOCAMPUS



DATA EXPRESSED AS MEANS WITH STANDARD ERRORS *p<0.05 STUDENT'S T-TEST

FIG. 11

IL-1 β CONCENTRATION IN SINGLE CELL SUSPENSION



VALUES ARE EXPRESSED AS THE MEAN WITH STANDARD ERROR *p<0.05 STUDENT'S T-TEST

FIG. 12

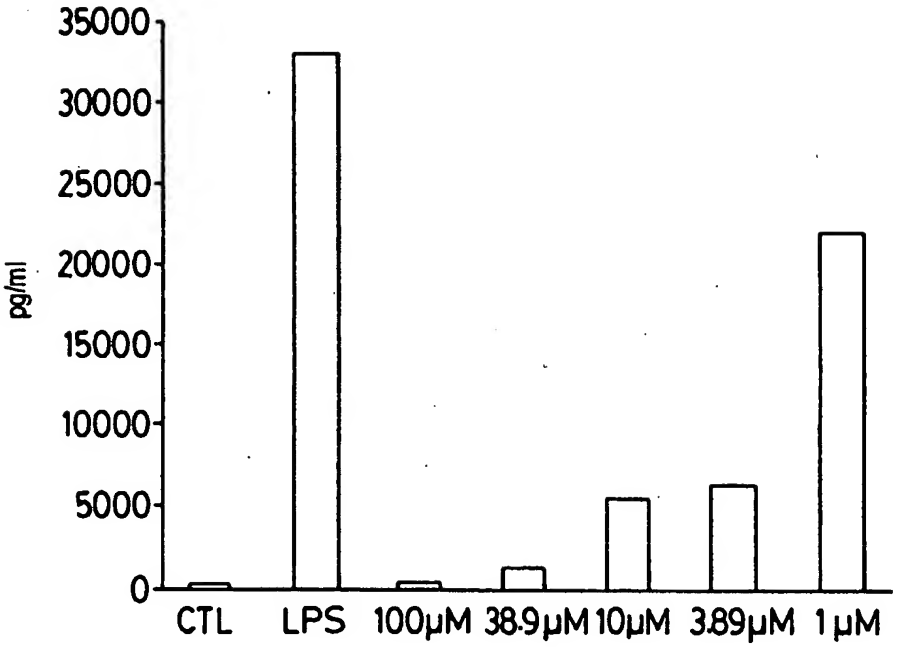

35000
30000
25000
20000
15000
10000
5000
0
pg/ml
CTL
LPS
100µM
38.9µM
10µM
3.89µM
1µM

FIG. 13

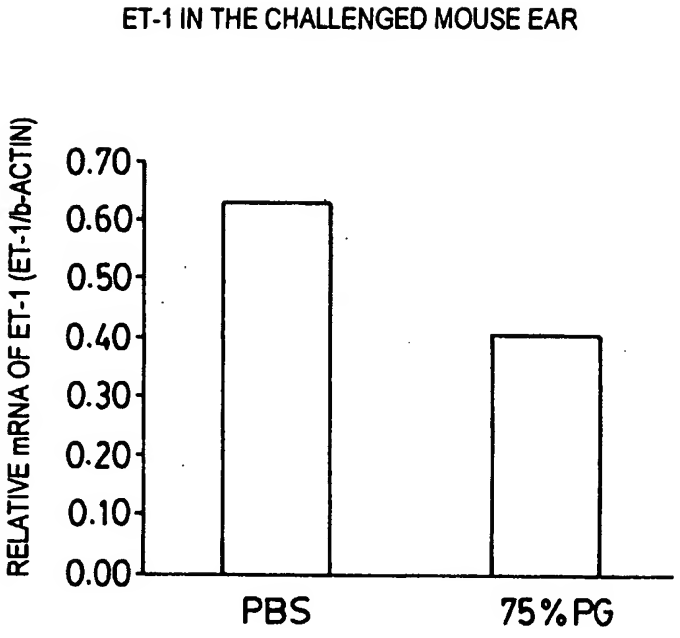

ET-1 IN THE CHALLENGED MOUSE EAR
RELATIVE mRNA OF ET-1 (ET-1/b-ACTIN)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
PBS
75% PG

FIG. 14

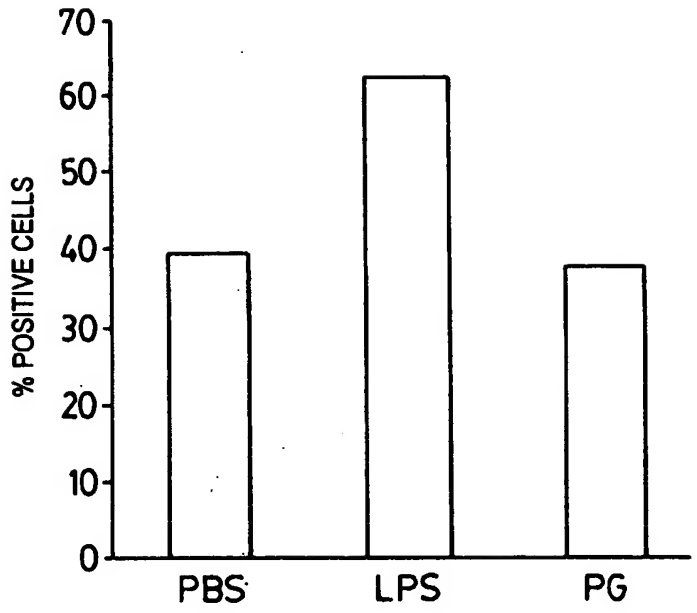


FIG. 15

INTERNATIONAL SEARCH REPORT

Internat[redacted] Application No
PCT/CA 03/00065

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/683 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 11781 A (ALCON LAB INC) 18 October 1990 (1990-10-18) page 5, paragraph 4 page 7, paragraph 1 examples 2,3 ---	1-51
X	WO 95 23592 A (UNIV BRITISH COLUMBIA) 8 September 1995 (1995-09-08) page 6, line 5-25 page 11, line 21-29 page 12, line 3-6 claims 1,4-6 ---	1-9, 25-33, 44-51
X	US 5 262 405 A (GIROD-VAQUEZ SOPHIE ET AL) 16 November 1993 (1993-11-16) column 5, line 2-16; claims 1,3 --- -/--	1-9, 44-51

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 April 2003	19/05/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vermeulen, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/CA 03/00065

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HAMMEL MICHAL ET AL: "Mechanism of the interaction of beta2-glycoprotein I with negatively charged phospholipid membranes." 27 November 2001 (2001-11-27) , BIOCHEMISTRY, VOL. 40, NR. 47, PAGE(S) 14173-14181 XP002237429 ISSN: 0006-2960 page 14173, paragraph 1 abstract	1-51

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat[redacted] Application No
PCT/CA 03/00065

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		US	2002110588 A1	15-08-2002
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		CA	2036629 A1	21-08-1991
		DE	69113848 D1	23-11-1995
		DE	69113848 T2	25-04-1996
		DK	450991 T3	04-03-1996
		EP	0450991 A2	09-10-1991
		JP	4211014 A	03-08-1992